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‘‘EVALUATION OF ANTIANGIOGENESIS ACTIVITY OF MYRISTICA MALABARICA LEAVES.

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI - 32.

In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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Under the Guidance of

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DEPARTMENT OF PHARMACOLOGY

J.K.K. NATTRAJA COLLEGE OF PHARMACY

Komarapalayam – 638183.

Tamil Nadu.

OCTOBER -2017

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"Evaluation of antiangiogenesis activity of *Myristica malabarica* leaves"'**submitted to "The TamilnaduDr.M.G.R. Medical University",Chennai.in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy in Pharmacology**,is a bonafide work carried out by **Mrs .RAFEEKA ABDUL RASSAK, Reg.No-261525207**,during the academic year 2016-2017, under my guidance and direct supervision in the department of pharmacology, J.K.K.Nataraja College of Pharmacy, Komarapalayam.

Internal Examiner **External Examiner**

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DECLARATON

I hereby declare that the dissertation **"Evaluation of antiangiogenesis activity of *Myristica malabarica* leaves "**, has been carried out under the guidance and supervision of Mr.V.Vvenkateswaran,M.Pharm, Assistant Professor, Department of Pharmacology,J.K.K.Nataraja College of Pharmacy.Komarapalayam, in partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmacology during the academic year 2016-2017.

I further declare that,this work is originaland this dissertation has not been submitted previously for the award of any other degree,diploma associate ship and fellowship or any other similar title.

Place :Komarapalayam,
Date:

RAFEEKA ABDUL RASSAK,
Reg.No:261525207,

Dedicated to

Parents,

Teachers&

My Family

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Mrs. RAFEEKA ABDULRASSAK
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1. INTRODUCTION

In The great advances of modern scientific medicine, traditional medicine is still the primary form of treating diseases of majority of people in developing countries including India. even among those to whom western medicine is available, the number of people using one or another of complementary of alternative medicine is rapidly increasing worldwide. Increasing knowledge of metabolic process and the effect of plants on human physiology has enlarged the range of application of medicinal plants .

Medicinal plants are a large group of plants used in medicine or veterinary practices for therapeutic or prophylactic purposes. They have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total.

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values.

Mainly in three ways medicinal plants has been found their use in medicine;

- They used directly or their natural chemical constituents are used
- They used as an agent in the synthesis of drugs
- Their organic molecules are used as a model for synthesis of drugs

Therapeutic properties of medicinal are identified mainly by the presence of alkaloids, glycosidess, flavanoids, vitamins, tannins, or coumarin derivatives in their organs.

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemical are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites compounds which are found in a range of plants, serving a more specific function. Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs.

Alkaloids are a class of chemical compounds containing a nitrogen ring. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the antihypertension agent reserpine.

Polyphenols (also known as phenolics) are compounds contain phenol rings. Glycosides is a molecule in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important roles in living organisms.

Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, which are often strong smelling and thus may have had a protective function. They are the major components of resin, and of turpentine produced from resin.

1.1 Characteristics of Medicinal Plants

Medicinal plants have many characteristics when used as a treatment, as follow:

- Synergic medicine- The ingredients of plants all interact simultaneously, so their uses can complement or damage
- others or neutralize their possible negative effects.

- Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.
- Preventive medicine- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment.

There are approximately half million plants with flowers, most of which have been investigated and which principles could be decive in the treatment of present or future diseases.

India is surrounded by water on its three sides, the west coast consist of the Western Ghats extending from Tapti in Gujarat to Kanniyakumari in Tamil Nadu including Maharashtra, Goa, Karnataka and Kerala. The Western Ghats are stretch of hill ranges which are known to be rich in flora and fauna, comprising about 17000 species of flowering plants are estimated in India of which, 4500 species a families of flowering plants comprising 19 genera and 400 species widely distributed, among which the genus comprises 80 species (Mabberley DJ, 1987). There are five species of excluding the cultivated M. fragrans in the Western Ghats region of Karnataka. They are attenuata, M. malabarica var. magnifica, M. malabarica in Myristica swamp forest, which is the habitat of this species. Due to anthropological activity and conversion of this habitat to cash crop plantations and teak forests, the fresh water swamp now dwindled to small fractions in Karnataka (Rama Bhat P. and Kaveriappa KM, 2009). M. malabarica Houtt. var. magnifica (Beddome) Sinclair is a lofty tree up to 30 m tall, trunk when young furnished with large aerial roots from the trunk. Leaves oblong to elliptic, rounded at base, acute to acuminate at ape 1a). Flowers in clusters, small, deciduous, ovoid, up to 2cm long densely rusty tomentose. Male flowering is 10 flowered cymes or umbels of 2-3cm long stamens 10. Female flowers are 4 (Rama Bhat P. and Kaveriappa KM, 1996. Fruits oblong, up to 10 cm long densely tomentose. Seeds subcylindric or ellipsoid, up to 5cm long, aril deeply much laciniate, orange is endemic, rare and threatened. It is commonly known as Choora pannu, as Dodda yele Ramapatre in Kannada, Kotthapanu or Kothapayin or

Churapayin in Malayalam. Ghats: Kerala- Kollam, Kozhikode, Thiruvananthapuram; Karn (Rama Bhat P. and Kaveriappa KM, 2009). India has an ancient heritage of traditional medicine. The on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicines are based on various system including Ayurveda, Siddha, Unani and Homeopathy. Plants are one of the most important sources of medicine. Today the large numbers of drugs i plants are rich in secondary metabolites and essential oils of therapeutic importance. The important advantages claimed PALGO JOURNAL OF MEDICINE AND MEDICAL SCIENCE .com Phytochemical screening, antimicrobial and antioxidant Myristica malabarica Houtt. var. magnifica Sinclair Medha Tantry and Rama Bhat P. PG Dept. of Biotechnology, Alva's College, Moodbidri – 574 227, Karnataka, India Accepted 10 October, 2016 The methanolic and aqueous seed extracts of Myristica malabarica var. magifica were obtained by soxhlet method and used for biochemical studies including antimicrobial and antioxidant activities using standard protocols. showed the presence of phytochemical constituents like carbohydrates, proteins, alkaloids resins whereas the aqueous extract showed the absence of phenolics, tannin and resin. Antimicrobial activities were bacterial species viz., Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella by agar well diffusion method. Out of two extracts, methanol extract were found to be more active against all the microorganisms than the aqueous extract. Antioxidant activity was determined by DPPH method which showed antioxidant potential of methanolic extract compared with the standard ascorbic acid. , antimicrobial activity, antioxidant activity, Myristica malabarica India is surrounded by water on its three sides, the west coast consist of the Western Ghats extending from Tapti in in Tamil Nadu including Maharashtra, Goa, Karnataka and Kerala. The Western Ghats are stretch of hill ranges which are known to be rich in flora and fauna, comprising about 17000 species of flowering plants are estimated in India of which, 4500 species are found in the Western Ghats. Myristicaceae is one of the important families of flowering plants comprising 19 genera and 400 species widely distributed, among which the genus comprises 80 species. There are five species of Myristicaceae belonging to three genera in the Western Ghats region of Karnataka. They are magnifica, M. malabarica and M.

dactyloides. M. malabarica var. magnifica swamp forest, which is the habitat of this species. Due to anthropological activity and conversion of this habitat to cash crop plantations and teak forests, the fresh water swamp now dwindled to small fractions in Karnataka ma Bhat P. and Kaveriappa KM, 2009). (Beddome) Sinclair is a lofty tree up to 30 m tall, trunk when young furnished with large aerial roots from the trunk. Leaves oblong to elliptic, rounded at base, acute to acuminate at ape 1a). Flowers in clusters, small, deciduous, ovoid, up to 2cm long densely rusty tomentose. Male flowering is 10 3cm long stamens 10. Female flowers are 4-6 flowered cymes or umbels of 1 ama Bhat P. and Kaveriappa KM, 1996. Fruits oblong, up to 10 cm long densely tomentose. Seeds subcylindric or ellipsoid, up to 5cm long, aril deeply much laciniate, orange-red (Plate 1b). Flowering and fruiting from March hreatened. It is commonly known as Choora pannu, as Dodda yele Ramapatre in Kannada, Kotthapanu or Kothapayin or Churapayin in Malayalam. It is distributed in as remnant patches in Southern Western Kollam, Kozhikode, Thiruvananthapuram; Karnataka-Uttara Kannada, Shimoga; Tamil Nadu. India has an ancient heritage of traditional medicine. The Materia Medica of India provides a great deal of information l aspects of therapeutically important natural products. Indian traditional medicines are based on various system including Ayurveda, Siddha, Unani and Homeopathy. Plants are one of the most important sources of medicine. Today the large numbers of drugs in use are derived from plants plants are rich in secondary metabolites and essential oils of therapeutic importance. The important advantages claimed Phytochemical screening, antimicrobial and antioxidant magnifica , Karnataka, India were obtained by soxhlet method and used for biochemical studies including antimicrobial and antioxidant activities using standard protocols. Methanolic extract proteins, alkaloids, tannins, phenolics and . Antimicrobial activities were Escherichia coli, Salmonella typhi, Klebsiella by agar well diffusion method. Out of two extracts, methanol extract were found to be than the aqueous extract. Antioxidant activity was determined by DPPH method which showed antioxidant potential of methanolic extract compared with the standard ascorbic acid. Myristica malabarica var. magnifica. India is surrounded by water on its three sides, the west coast consist of the Western Ghats extending from Tapti in in Tamil Nadu

including Maharashtra, Goa, Karnataka and Kerala. The Western Ghats are stretch of hill ranges which are known to be rich in flora and fauna, comprising about 17000 species of flowering plants re found in the Western Ghats. Myristicaceae is one of the important families of flowering plants comprising 19 genera and 400 species widely distributed, among which the genus Myristica Myristicaceae belonging to three genera in the Western Ghats region of Karnataka. They are G. farquhariana, Knema magnifica is dominant species found swamp forest, which is the habitat of this species. Due to anthropological activity and conversion of this habitat to cash crop plantations and teak forests, the fresh water swamp now dwindled to small fractions in Karnataka (Beddome) Sinclair is a lofty tree up to 30 m tall, trunk when young furnished with large aerial roots from the trunk. Leaves oblong to elliptic, rounded at base, acute to acuminate at apex, silvery beneath. Flowers in clusters, small, deciduous, ovoid, up to 2cm long densely rusty tomentose. Male flowering is 10-20 6 flowered cymes or umbels of 1-2cm long ama Bhat P. and. Fruits oblong, up to 10 cm long densely tomentose. Seeds subcylindric or red (Plate 1b). Flowering and fruiting from March-October. It hreatened. It is commonly known as Choora pannu, as Dodda yele Ramapatre in Kannada, It is distributed in as remnant patches in Southern Western Uttara Kannada, Shimoga; Tamil Nadu- Tirunelveli of India provides a great deal of information l aspects of therapeutically important natural products. Indian traditional medicines are based on various system including Ayurveda, Siddha, Unani and Homeopathy. Plants are one of the most important n use are derived from plants. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance.

Therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. The chemical compounds that occur naturally in plants and responsible for colour and organoleptic properties, such as deep purple of blue berries and smell of garlic are called photochemicals. These phytochemicals may have biological significance but are not established as essential nutrients. Scientists estimates that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer,

stroke or metabolic syndrome. These phytochemicals are abundant in fruits, vegetables and herbs. The use of plant extracts and phytochemicals, both with known antimicrobial properties, are of great significance to therapeutic treatments. Extracts of plants were used for the treatment of various diseases and this forms the basis for all Indian system of medicines. However this area is not much developed when compared to modern system of medicines, mainly because of the lack of scientific documentation in this field. Recent investigations revealed that plant origin antioxidants have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurogenerative diseases, cardiovascular diseases, aging, gastrointestinal diseases. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effect; while relatively plant based medicine confer fewer side effects than the synthetic drugs in some instances. Myristica malabarica var. magnifica belongs to the family Myristicaceae (nutmeg family). The other species of this family contains antimicrobial and antioxidant activity. There is one report on the use of aril covering the seed of this plant used as dyeing in Dakshina Kannada; the wood has no other uses. The above properties may be present in M. malabarica var. magnifica seeds and arils. So a detailed study is required for analysis. But exploitation of plant should be narrowed as the species is listed in the RED List of Plants which needs special care of conservation strategy. Conservation of habitat as well as fresh water ecosystem – the Myristica swamp needs first rank of attention in the biodiversity conservation acts. The seeds are of recalcitrant type, looses viability rapidly. Nutmeg (M. fragrans) contains many plant-derived chemical compounds that are known to have been anti-oxidant, disease preventing, and health promoting properties. The active principles in nutmeg have many therapeutic applications in many traditional medicines as anti-fungal, anti-depressant, aphrodisiac, digestive, and carminative functions. Alcoholic extract of nutmeg have antibacterial activity against Micrococcus pyrogenes var. aureus. Aqueous decoctions are toxic to cockroaches. Myristicin is used as an additive to pyrethrum to enhance its toxicity against houseflies. The leaf essential oils have weedicidal properties. It is also used for making soaps, dentifrices, chewing gums and tobacco. Nutmeg pericarp is used in pickles and jellies. Nutmeg oil contains eugenol, which has been used in dentistry for

toothache relief. The oil is also used as a local massage to reduce muscular pain and rheumatic pain of joints. Freshly prepared decoction with honey has been used to relief of nausea, gastritis, and indigestion ailments. On the other hand M. malabarica (wild nutmeg) seed used in external application for indolent ulcers, crude fat from seeds analgesic and used in rheumatism and gangrene. The yellowish maize is used as an adulterant for true mace. The seed and aril extracts possess antifungal and antibacterial activity. Based on the above observations a research study has been undertaken with the following objectives: To analyse the phytochemical constituents in the aqueous and methanolic extract of the seed of Myristica malabarica var. magnifica. To evaluate antimicrobial activity of methanolic and aqueous extract on selected bacterial and fungal strains. To study the antioxidant activity of aqueous and methanolic extracts of the seed of M. malabarica var. magnifica.

1.2 CANCER :

Cancer is a rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs or cancer is a generic term for a large group of disease that affect any part of the body. The abnormal cells are termed cancer cells, malignant cells, or tumor cells. It is a common condition and a serious health problem. Cancer is a leading cause of death worldwide. One in three people will develop some forms of cancer during their lifetime. Lung, stomach, liver, colon, and breast cancer cause the most cancer deaths each year. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030. Cancer is not confined to humans; animals and other living organisms can get cancer. . Cancer, in medicine, common term for neoplasms, or tumors, that is malignant. Like benign tumors, malignant tumors do not respond to normal body mechanism, that controls normal cell growth. Malignant tumors consist of undifferentiated, or unspecified, cells that show an atypical cell structure and do not function like the normal cells from the organ from which it derives. Cancer cells lack contact inhibition. Loss of contact inhibition accounts for two other characteristics of cancer cells; invasiveness of surrounding tissues, and metastasis, or spreading via lymphatic system or blood to other tissues and organs.

Normal cells have a limited lifespan controlled by the telomere gene, which signals the end of cell life. Cancer cell contains telomerase, an enzyme that alters the telomere gene and allow the cell continue to divide. Cancers tissue, growing without limits, competes with normal tissue for nutrients, eventually killing normal tissue by nutritional deprivation. Cancerous tissue also cause secondary effects, in which the expanding malignant growth puts pressure on surrounding tissue or organs or the cancer cells that metastasis and invade other organs.21

1.3 CLASSIFICATION [7]

There are over 200 types of cancers; most can fit into the following categories. Cancers are classified by the type of cell that the tumor cells resemble and are therefore presumed to be the origin of the tumor. These types include:

- Carcinoma: Cancers derived from epithelial cells.
- Sarcoma: Cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develop from cells originating in mesenchymal cells outside the bone marrow.
- Lymphoma and leukemia: These two classes of cancer arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively.
- Germ cell tumor: Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).
- Blastoma: Cancers derived from immature "precursor" cells or embryonic tissue

1.4 SIGNS AND SYMPTOMS OF CANCER [8,9].

Symptoms and signs of cancer depend on the type of cancer, where it is located and capacity to spread. However, below specified signs and symptoms were the indication of cancer.

- Fever (no clear infectious source, recurrent or constant)
- Fatigue (not relived by rest)
- Weight loss (without trying to lose weight)
- Pain (usually persistent)
- Skin changes (coloration, sores that do not heal, white spots in mouth or on tongue, wart changes)
- Change in bowel or bladder functions (including trouble swallowing or constipation)
- Unusual bleeding (mouth, vaginal, and bladder) or discharge
- Persistent cough or change in voice
- Lumps or tissue masses

1.5 DIAGNOSIS OF CANCER [10,11]

Most cancers are initially recognized either because of the appearance of signs or symptoms or through screening. People with suspected cancer are investigated with medical tests. These commonly include blood tests, X-rays, CT scans and endoscopy. Imaging studies are commonly used to help physicians detect abnormalities in the body that may be cancer. The biopsy can provide more than the definitive diagnosis of cancer; it can identify the cancer type (for example, a primary or metastatic type of brain cancer) and the "stage" of the cancerous cells.

Cancer treatment depends on the type of cancer, the stage of the cancer (how much it has spread), age, health status, and additional personal characteristics. There is no single treatment for cancer, and patients often receive a combination of therapies and palliative care [12,13]. Treatments usually fall into one of the following categories:

- Surgery
- Radiation
- Chemotherapy
- Immunotherapy
- Hormone therapy

- Gene therapy

1.6 SURGERY

Surgery is the oldest known treatment for cancer. If a cancer has not metastasized, it is possible to completely cure a patient by surgically removing the cancer from the body. This is often seen in the removal of the prostate or a breast or testicle.

1.7 RADIATION[14]

Radiation therapy involves the use of ionizing radiation in an attempt to either cure or improve the symptoms of cancer. It is used in about half of all cases and the radiation can be from either internal sources in the form of brachytherapy or external sources. Radiation is typically used in addition to surgery and or chemotherapy but for certain types of cancer such as early head and neck cancer may be used alone.

1.8 IMMUNOTHERAPY

Immunotherapy aims to get the body's immune system to fight the tumor. Local immunotherapy injects a treatment into an affected area, Systemic immunotherapy treats the whole body by administering an agent such as the protein interferon alpha that can shrink tumors.

1.9 HORMONE THERAPY

Several cancers have been linked to some types of hormones, most notably breast and prostate cancer. Hormone therapy is designed to alter hormone production in the body so that cancer cells stop growing or are killed completely.

1.10 GENE THERAPY[15]

The goal of gene therapy is to replace damaged genes with ones that work to address a root cause of cancer: damage to DNA. . Other gene-based therapies focus on further damaging cancer cell DNA to the point where the cell commits suicide. Gene therapy is a very young field and has not yet resulted in any successful treatments.

1.11 PALLIATIVE CARE [16]

Palliative care refers to treatment which attempts to make the patient feel better and may or may not be combined with an attempt to attack the cancer. Palliative care includes action to reduce the physical, emotional, spiritual, and psycho-social distress experienced by people with cancer. Unlike treatment that is aimed at directly killing cancer cells, the primary goal of palliative care is to improve the patient's quality of life.

1.12 PREVENTION OF CANCER [17]

Cancer prevention is defined as active measures to decrease the risk of cancer. The vast majority of cancer cases are due to environmental risk factors, and many, but not all, of these environmental factors are controllable lifestyle choices. Thus, cancer is considered a largely preventable disease. Greater than 30% of cancer deaths could be prevented by avoiding risk factors including: tobacco, overweight / obesity, an insufficient diet, physical inactivity, alcohol, sexually transmitted infections, and air pollution[18].

1.13 MEDICATION [19]

The concept that medications can be used to prevent cancer is attractive, and evidence supports their use in a few defined circumstances. In the general population NSAIDs reduce the risk of colorectal cancer however due to the cardiovascular and gastrointestinal side effects they cause overall harm when used for prevention. Aspirin has been found to reduce the risk of death from cancer by about 7%. COX-2 inhibitor may decrease the rate of polyp formation in people with familial adenomatous polyposis however are associated with the same adverse effects as NSAIDs. Daily use of tamoxifen or raloxifene has been demonstrated to reduce the risk of developing breast cancer in high-risk women. Vitamins have not been found to be effective at preventing cancer, although low blood levels of vitamin D are correlated with increased cancer risk.

1.14 VACCINATION

Certain vaccinations have been associated with the prevention of some cancers. For example, many women receive a vaccination for the human papillomavirus because of the virus's relationship with cervical cancer. Hepatitis B vaccines prevent the hepatitis B virus, which can cause liver cancer.

From modality of administration of herbal medication, anti-cancer herbal medicines are herein classified into 3 groups, including take by mouth, topical use and injectable preparations(not available at our HCC and HMC)[20]. Since1990, a research group of hi-tech herbal medicine directed by Dr. David Liu has conducted many studies on herbal treatments in chronic diseases and tumors. We have screened all 66 sorts of anti-cancer medicinal herbs using our unique techniques, including identify and analysis of ester-soluble active ingredients , ethanol-soluble active ingredients and hydrate-soluble active ingredients in each potential anti-cancer medicinal herb or plant[21].

1.15 RECENT RESEARCH AND DEVELOPMENTS

1.15.1 ANGIOGENESIS AND ANTIANGIOGENESIS [22]

For the metastatic spread of cancer tissue, growth of the vascular network is important. The process whereby new blood vessels form is called angiogenesis. Angiogenesis is the physiological process through which new blood vessels form from preexisting vessels. The essential role of angiogenesis in tumor growth was first proposed by Judah Folkman1971. Angiogenesis has an essential role in the formation of a new vascular network to supply nutrients, oxygen and immune cells and also remove waste products. Angiogenesis has been correlated with disease progression and poor prognosis in many tumor types which can be activated at different stages of tumor development, depending on the tumor type and micro environmental conditions. Tumor growth and metastasis depends on angiogenesis triggered by chemical signals from tumor cells in a phase of rapid growth. Without angiogenesis cancer cells can’t grow beyond 2cumm or it can grow beyond 2cumm when

angiogenesis is possible. In the absence of vascular support tumors may become necrotic or even panoptic. Therefore angiogenesis is an important factor in the progression of cancer. Angiogenesis basically includes a four step process. First, the basement membrane in tissues is injured locally due to the interaction between tissue and carcinogens, which leads to immediate destruction and hypoxia. Second angiogenesis factors migrate?. Third, endothelial cells proliferate and stabilize and fourth is angiogenesis factor continue to influence the antigenic process. Angiogenesis is regulated by a balance between activators and inhibitors [23].

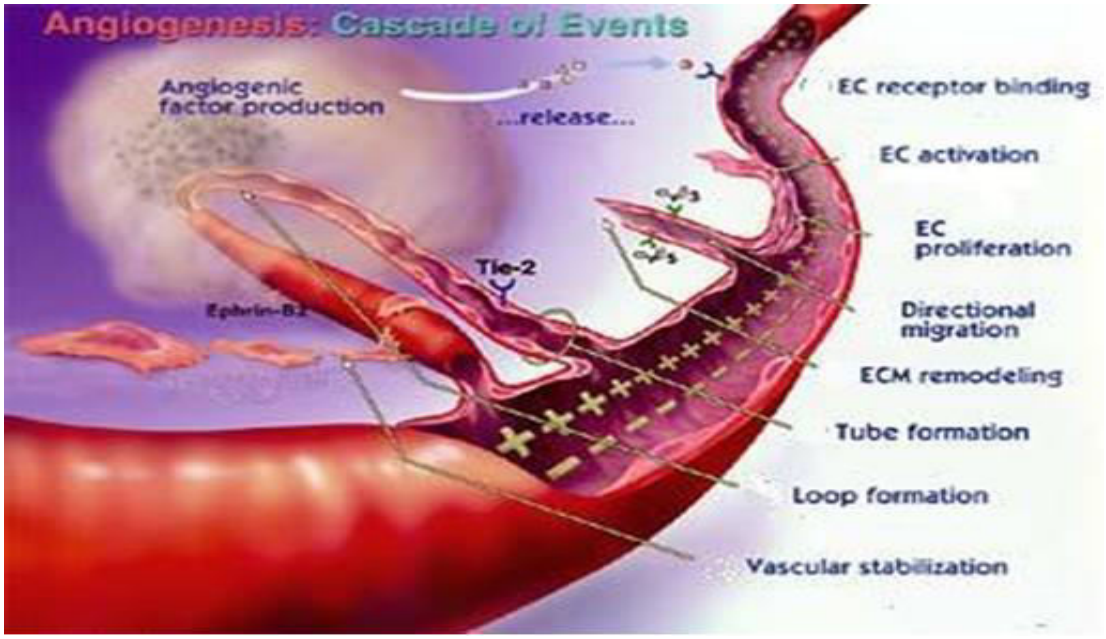
If angiogenesis is so critical for the tumor growth, then the agents that inhibit angiogenesis would have great therapeutic value. With the discovery of endostatin, the concept of anti-angiogenesis was launched. As early as the 1970s, Dr. Judah Folkman of the Harvard medical school suggested inhibiting new blood vessel formation as a way to fight cancer. Angiogenesis inhibitors can be discovered from a variety of sources. Some are naturally present in human body which fragments of structural proteins such as collagen or plasminogen. Others are natural products in green tea, soyabeans, fungi, mushrooms, tree barks, shark tissues, snake venom etc [24].

Recent research and developments is expressed biologically relevant data about the angiogenisis and anti angiogenisis treatments in the modern methods of medicinal practices-angiogenesis based Medicine which are restoring the body's natural control of angiogenesis, it is a new, comprehensive approach to fighting against disease. By using new medical treatments that either inhibit or stimulate angiogenesis, doctors are prolonging the lives of cancer patients, preventing limb amputations, reversing vision loss and improving general health. In the synaryom of all cancerous tumors, for example, release angiogenic growth factor proteins that stimulate blood vessels to grow into the tumor, providing with oxygen and nutrients [25]. Antiangiogenic therapies literally starve the tumor of its blood supply by interfering with this process. A new class of cancer treatments that block angiogenesis are now approved and available to treat cancers of the colon, kidney, lung, breast, liver, brain, and thyroid, as well as multiple myeloma, bone gastrointestinal stromal

tumors, soft tissue sarcoma, and SEGA tumors. Some older drugs have been rediscovered to block angiogenesis, as well. These are being used to treatment angiogenesis-dependent conditions, such as hemangiomas, colon polyps, and precancerous skin lesions [26].

Therapeutic angiogenesis, in contrast, stimulates angiogenesis where it is required but lacking. This technique is used to replenish the blood supply to chronic wounds to speed healing, and it prevents unnecessary amputations [27]. New research suggests this approach can be also used to save limbs afflicted with poor circulation, and even oxygen-starved hearts. Therapeutic angiogenesis may even help to regenerate damaged or lost tissues in ways that were previously considered impossible, such as with nerves and brain tissue. In this data clearly mentioned about the importance of the angiogenesis and anti angiogenesis in modern medicinal practices [28]. Recently some literatures indicated different types of angiogenesis available such as sprouting and intususceptive[29&30].

DIFFERENT STAGES OF ANGIOGENESIS



ANTIANGIOGENESIS DRUGS

The steps involved in pathway are a possible target for cancer treatment. Different drugs may works at different step in this pathway.

- Inhibiting endogenous angiogenic factors , such as BFGF and VEGF.
- Inhibiting degenerative enzymes (matrix metalloproteinase) responsible for the degradation of the basement membrane of blood vessels.
- Inhibiting endothelial cell proliferation.
- Inhibiting endothelial cell migration.
- Inhibiting activation and differentiation of endothelial drugs[31]

Angiogenesis Inhibitors in Clinical Trials for Cancer

In part from National Cancer Institute Database **(Updated March 2001)**

Phase I

Drug	Sponsor	Mechanism
SU6668	Sugen	Blocks VEGF, FGF & EGF receptor signaling
Endostatin	EntreMed	Inhibits endothelial proliferation
Angiostatin	EntreMed	Inhibits endothelial proliferation
Combretastatin	Oxigene	Apoptosis in proliferating endothelium
IMC-1C11	ImClone	Monoclonal antibody to KDR receptor
ZD6474	AstraZeneca	Inhibits VEGF receptor-associated tyrosine kinase

Phase II

CAI	NCI	Inhibits calcium influx
COL-3	Collagenex, NCI	Synthetic MMP inhibitor; tetracycline derivative
Squalamine	Magainin	Inhibits Na/H exchanger
TNP-470	TAP Pharm.	Fumagillin analogue; inhibits endothelial proliferation
2-methoxy-estradiol (Panzem)	EntreMed	Inhibits microtubule function
Interleukin-12	Genetics Inst.	Induces IFN-gamma and IP-10
EMD 121974	Merck KCgaA	Blocks an endothelial integrin
Anti-VEGF Ab	Genentech	Monoclonal antibody to VEGF
Prinomastat	Agouron	Synthetic MMP inhibitor

Phase III

Marimastat	British Biotech	Synthetic MMP inhibitor
Neovastat	Aeterna	Natural MMP inhibitor
Interferon-alfa	Commercially available	Inhibition of bFGF production
IM862	Cytran	Endothelial inhibitor
Thalidomide	Celgene	Unknown
SU5416	Sugen	Blocks VEGF receptor signaling
BMS-275291	Bristol-Myers Squibb	Synthetic MMP inhibitor

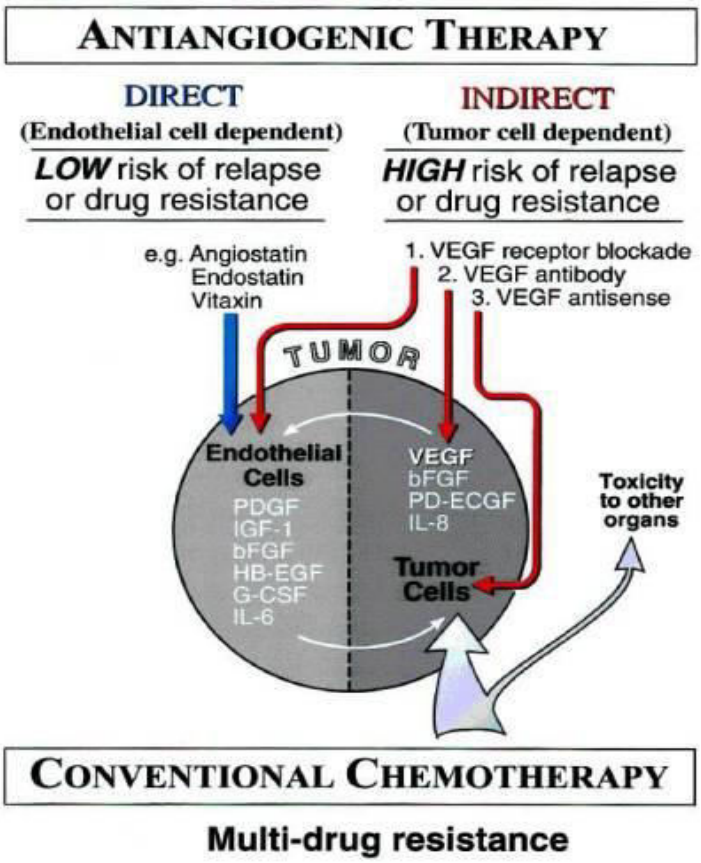
SIDE EFFECTS OF ANTI ANGIOGENIC DRUGS [32]

Angiogenic drugs have milder side effects than chemotherapy drugs. Anti angiogenic drugs are fairly new, it's not yet clear if the effects see so far will be seen with all the drugs.

- Bleeding or holes in digestive tract
- Raised blood pressure
- Surgery risks

- Pregnancy risks

Newer approaches that combine anti angiogenic drugs with chemotherapy , other targeted drugs, or radiation may work better than using them alone.



2. LITERATURE REVIEW

Banerjee Dmaity et.al.,2016 Investigated and reported The healing activity of the methanol extract of the *Myristica malabarica* and omeprazole against indometacin-induced stomach ulceration in a mouse model. Treatment with RM (40 mg kg(-1) per day) and omeprazole (3 mg kg(-1) per day) for 3 days could effectively heal the stomach ulceration, as revealed from the ulcer indices and histopathological studies. Compared with the ulcerated group, treatment with RM and omeprazole for 3 days reduced the macroscopic damage score by approximately 72% and 76%, respectively ($P<0.001$), establishing the efficacy of RM. The extent of ulcer healing offered by 3 days' treatment with RM or omeprazole was better than that observed with natural recovery over 5 and 7 days ($P<0.05$). The healing capacities of RM and omeprazole could be attributed to their antioxidant activity as well as the ability to enhance the mucin content of the gastric tissues. Both drugs reduced lipid peroxidation (by 42-44%) and protein carbonyl content (by 34%), and augmented non-protein thiol levels beyond normal values. Furthermore, RM improved the mucin level beyond the normal value, while omeprazole restored it to near normalcy.

Biswanath Maity et.al 2016 Studied and reported the 3rd day of its administration to mice, indomethacin (18 mg kg^{-1}, *p.o.*) produced maximum stomach ulceration with a damage score of 3.46, which was reduced by a 3-day treatment with the methanol extract of *Myristica malabarica* (40 mg kg^{-1}, *p.o.*) and omeprazole (3 mg kg^{-1}, *p.o.*) to 0.95 and 0.82, respectively. Presently, they investigated the possible role of the test samples in modulating PG synthesis and angiogenesis for their healing action. The ulceration was found to be associated with suppression of PGE_2, VEGF and vWF VIII, and an increase in EGF and endostatin levels. Treatment with the plant extract reversed all these parameters accounting for its healing activity. However, despite providing similar healing, omeprazole did not alter these parameters.

Babu et.al 2010 Studied the Biochemical aspects of desiccation induced viability loss in *myristica malabarica* by exposing freshly collected mature seeds. At the room

temperature 28± 2°C, and 60%RH.)Moisture content and germination rate were reduced uniformly and viability (72%) was retained up to 6 days when the moisture content was reduced to one half. Electrolyte leakage and lipid peroxidation showed linear increase while formozan intensity was reduced gradually until the loss of viability. Peroxidase andpolyphenoloxidase were more active up to 4th day of desiccation compared to the control whereas, drastic reduction in the activity of these enzymes was observed coinciding with loss of viability. Even though *Myristica malabarica* seeds containedonly 27% moisture content and were considered as moderately recalcitrant because these seeds were highly sensitive todesiccation and the loss of viability begans after one day and ends within 7-8days. The desiccation sensitivity appeared to be due to manifold electrolyte leakage and lipid peroxidation and comparatively reduced enzymatic protection expressed asperoxidase and polyphenol oxidase against free radicals formed due to desiccation stress.

Bauri et.al 2015 isolated the methanol extract of *myristica malabarica* and reported The title compound, malabaricone A [systematic name: 1-(2,6-dihydroxyphenyl)-9-phenylnonan-1-one], $C_{21}H_{26}O_3$, contains two benzene rings linked through a C_9 alkyl chain. Both intra- and intermolecular O-H hydrogen-bonding interactions stabilize the packing. The intermolecular hydrogen bonds result in the formation of an infinite zigzag chain.

Choi Nam Hee-Kwon et.al 2008 Isolated the active constituent of malabaricones from the medicinal plant of *myristica malabarica* then investigated about the nematicidal activity against bursaphelenchus xylophilus and reported. *Bursaphelenchus xylophilus*,is the causative agent of pine wilt disease. the methanol extract of *Myristica malabarica* fruit rinds showed the most potent nematicidal activity with mortality of 100% at a concentration of 1000 μg ml^{-1} against *B. xylophilus*. Three nematicidal substances were isolated by bioassay-guided fractionation and then identified as malabaricones A, B and C by mass and NMR spectral data. Both malabaricones B and C showed higher activity than malabaricone A. There was a significant synergistic interaction between the three compounds on the pine wood nematode. When the combination of three compounds at a ratio of 1 : 1 : 1 was

applied, its EC_{50} value was 11.8 μg ml^{-1}. These results indicate that the extract of *M. malabarica* containing the three resorcinols could be useful as a natural nematicide for the control of pine wilt disease.

Consolacion Ragasa et.al 2016 Isolated the resorcinol from the plants of spiecies myristicaceae including *myristica malabarica*.The dichloromethane extract of the air-dried leaves of Myristica philippensis afforded two resorcinols, 1 and 2, and β-sitosterol. The structure of1 was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by mass spectrometry and comparison of its 13C NMR data with those of malabaricone C . The structure of 2 was deduced by comparison of its 1HNMR data with 1 and confirmed by comparison of its 13C NMR data with those of malabaricone B . β-Sitosterol was identified by comparison of its 1H NMR data with those reported in the literature . The resorcinols, malabaricone C and malabaricone B isolated from M. philippensis have been previously reported as constituents of M. fragrans , M. maingayi , M. gigantea , M. dactyloides and M. crassa , while he was also reported as a constituent of M. malabarica and M. cinnamomea . Thus, these resorcinols which were reported to exhibit various biological activities are found in the genus Myristica of the family Myristicaceae.

Indira iyer et.al 2008 Studied the direct somatic embryogenesis was obtained from intact and fragmented zygotic embryos of *Myristica malabarica*, an endemic, threatened medicinal species of Western Ghats of Southern India while cultured in Murashige and Skoog medium containing activated charcoal. In the absence of activated charcoal there was no embryogenic response but only callus formation in zygotic embryos and their fragments. The addition of gibberellic acid resulted in the emergence of shoot from the somatic embryos. The various developmental stages of somatic embryos were examined using scanning electron microscope. Thin layer chromatography revealed the presence of compounds similar to lignans in the embryogenic mass. GC-MS analysis of the embryogenic mass revealed the presence of several compounds of potential clinical value including malabaricone A , α-spinasterol and γ- sitosterol. The spent medium showed strong anti-bacterial activity against *Pseudomonas aeruginosa*. The results are significant since this is the first

report of tissue culture and induction of somatic embryogenesis in *Myristica malabarica*. The embryogenic culture system is potentially useful for efficient and consistent production of bioactive compounds and also has strong implications for conservation of its valuable germplasm.

John Zachariah et.al 2006 Reported the Essential oil constituents of leaves of three Myristica species namely, Myristica beddomeii, M. fragransand M. malabarica were determined by gas chromatography and gas chromatography-massspectrometry. M. fragrans was dominated by monoterpenes (91%), M. beddomeii contained mono- (48%) and sesquiterpenes(35%) whereas M. malabarica was dominated by sesquiterpenes (73%).The leaf oil of M. beddomeii was dominated by α-pinene (19.59%), t-caryophyllene (14.63%) andβ-pinene (12.46%). The leaf oil of M. fragrans contained sabinene (19.07%), α-pinene (18.04%), 4-terpineol (11.83%), limonene (8.32%) and β-pinene (7.92%) as major compounds, while t-caryophyllene (20.15%), α-humulene (10.17%), nerolidol (9.25%) and δ-cadinene (6.72%) werepredominant in the oil of M. malabarica. Linalool, α-terpineol, t-caryophyllene, β-elemene and γ-elemene were present in all the three species. This is the first report on the essential oil compositionof M. beddomeii leaves.

Kristi Dover et.al 2015 reviewed the pathophysiologic rationale and therapeutic applications of inhibiting angiogenesis in solid tumor growth.he investigated and reported the anti angiogenisis therapy is the useful and meaningful treatment option for the controlling malignant growth. Angiogenesis, the formation of new blood vessels, is necessary for the development of significant solid tumor growth. Inhibition of angiogenesis is a unique mechanism of antineoplastic therapy that does not use traditional cytotoxic actions. Four investigational antiangiogenic agents are currently being evaluated in phase I and II trials. Potentially beneficial applications of angiogenesis inhibitors include suppression of occult and premalignant lesions, symptomatic control of angiogenesis-dependent malignancies, and combination therapy with traditional antineoplastic agents. **Finally he concluded that** Inhibition of angiogenesis is a new pharmacologic strategy that may powerful in controlling malignant growth. A number of agents with antiangiogenic activity have been

developed, and further study of these drugs will define their role in antineoplastic therapy.

Manjunatha et.al 2011 Investigated and reported the antioxidant activities of the ethanol extract of myristica malabarica. Benzene and chloroform fraction from seed aril of *Myristica malabarica* (Myristicaceae) were assessed in an effort to validate the hepatoprotective potency of this plant. The extract and the fractions showed scavenging of 1, 1-diphenyl-2picrylhydrazyl (DPPH) radical and inhibition of ABTS radical in vitro. Antioxidant activities of the extracts were also demonstrable in vivo by the inhibition of the carbon tetrachloride (CCl) - induced formation of lipid peroxides in the liver of rats by 4 pretreatment with the extracts. CCl - induced hepatotoxicity in rats, as judged by the raised serum enzymes, 4 aspartate transaminase, alanine transaminase and alkaline phosphatases was prevented by pretreatment with the extracts/fractions, demonstrating the hepatoprotective action. Among the tested extracts benzene fraction recorded highest efficiency in protecting liver damage induced by toxic effect of CCl followed by crude 4 ethanolic extract and chloroform fraction. These findings were confirmed by histopathological study of the liver sections of the treated groups.

Nam Hee Choi et.al 2008 Investigated plant extracts with *in vivo* antifungal activity for plant diseases, he found that the methanol extract of *Myristica malabarica* fruit rinds effectively suppressed the development of several plant diseases. The methanol extract exhibited potent 1-day protective activity against rice blast, tomato late blight, wheat leaf rust and red pepper anthracnose. It also showed 7-day and 4-day protective activities against the plant diseases. Three antifungal resorcinols were isolated from the methanol extract of *Myristica malabarica* fruit rinds and identified as malabaricones A (MA), B (MB), and C (MC). Inhibitory activity of the three resorcinols against mycelia growth of plant pathogenic fungi varied according to compound and target species. All three compounds effectively reduced the development of rice blast, wheat leaf rust and red pepper anthracnose. In addition, MC was highly active for reducing the development of tomato late blight. This is the first report on the antifungal activities of malabaricones against filamentous fungi.

Patro et.al 2008 Reported the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay of the ether, methanol, and aqueous extracts of the medicinal plant *Myristica malabarica* (rampatri) revealed the methanol extract to possess the best antioxidant activity. Column chromatography of the methanol extract led to the isolation of a new 2-acylresorcinol and four known diarylnonanoids of which the diarylnonanoid, malabaricone C, showed the maximum DPPH scavenging activity. Malabaricone C could prevent both Fe(II)- and 2,2'-azobis(2-amidinopropane) dihydrochloride-induced lipid peroxidation (LPO) of rat liver mitochondria more efficiently than curcumin. The anti-LPO activity of malabaricone C was attributed to its better free radical scavenging and Fe(II) chelation capacities. The superior activity of malabaricone C was rationalized by a systematic structure-activity correlation of the results obtained with the structurally related diarylnonanoids and curcumin. Malabaricone C also prevented the gamma-ray-induced damage of pBR322 plasmid DNA in a concentration-dependent manner. The radioprotective activity was found to correlate with its OH radical scavenging property, which matched well with that of d-mannitol.

Purushothaman et.al 2016 isolated Malabaricones A--D, novel diarylnonanoids from Myristica malabarica Lam (Myristicaceae).He was reported Four novel diarylnonanoids, malabaricones A–D, have been isolated from the fruit rind of Myristica malabarica Lam (Myristicaceae) and assigned the structures (1)–(4).

Rupashree Sen et.al 2007 studied the antileishmanial activity of the fruit rind of *myristica malabarica.* that is used as a spice and is also credited with medicinal properties. The antipromastigote activity of different extracts/fractions of *Myristica malabarica* and its constituent diarylnonanoids were evaluated in *Leishmania donovani* promastigotes (MHOM/IN/83/AG83) using the MTS-PMS assay. Preliminary screening of the ether extract (R1) with its crude methanol fraction (R2) and two fractions (R3 and R4) revealed that R2 had potent leishmanicidal activity (IC_{50} 31.0 µg/mL), whereas R3 and R4 showed poor activity. Fractionation of R2 yielded four diarylnonanoids (malabaricones A–D, designated as Mal A, Mal B, Mal C and Mal D, respectively). The IC_{50}values of Mal A–D were 16, 22, 27 and >50

µg/mL, respectively. Taken together, the data suggest that the methanol extract of *Myristica malabarica*, especially its constituent compounds, Mal A and Mal B, have promising anti leishmanial activity meriting further investigations regarding the underlying molecular mechanism(s) of action with a view towards future drug development.

Ramabhati et.al 2007 Conducted ecological studies on Myristica swamp forests in Uttara Kannada district of Karnataka state in India with reference to floristic composition, structure, diversity and edaphic factors were conducted. Transect method was employed by laying out sample plots in Kathalkane Reserve Forests of Western Ghats and enumeration of all trees >10 cm diameter at breast height was made. Sixty three species, including one unidentified species of trees and bamboos belonging to twenty six families with DBH >10 cm were recorded. The forest is of evergreen type dominanted by Myristica fatua var. magnifica, Gymnacranthera farquhariana, Hopea ponga and Dipterocarpus indicus. Myristicaceae dominated the swamps with maximum Importance Value Index of 102.63 represented mainly by G. farquhariana (57.83) and M. fatua var. magnifica (38.49). The forest floor is covered by knee roots. The physico-chemical properties of the soil of swamp were also determined. Endemism was fairly high, with 23 species endemic to the Western Ghats and ten species were rare and threatened. Soils were silty and sandy loam of acidic to neutral pH and moderate organic carbon levels. Soil nitrogen, phosphorous and potassium contents were in theranges 0.64-1.26%, also slightly lower than other forest ecosystems of the region.

Ribatti et.al 2008 Reported the Chick embryo chorioallantoic membrane is one of useful method of screening the angiogenesis and antiangiogenesis activity. The chick embryo chorioallantoic membrane (CAM) is an extraembryonic membrane mediating gas and nutrient exchanges until hatching. Because it has a dense capillary network, it has been commonly used in vivo to study both angiogenesis and antiangiogenesis in response to normal tissues and cells, to tumor bioptic specimens and cells, or to soluble factors. During the last 8 years, this assay has been used in over 550 published works. The angiogenic response of CAM to multiple myeloma and neuroblastoma

cells and bioptic specimens and their responses to antiangiogenic molecules and the role played by fibroblast growth factor-2 in CAM vascularization have been analyzed in detailed manner and published .

Sabulal Baby et.al 2007 identified and reported the chemical composition of the leaf oils of *myristica malabarica* (Myristicaceae) were isolated by hydrodistillation and analyzed by GC/FID and GC/MS. Seventy-six constituents (98.5%) with β-caryophyllene (27.3%), α-humulene (13.8%) and α-copaene (11.5%) as major components were identified from the leaf oil of *Myristica malabarica*. Sesquiterpene hydrocarbons constituted 77.3% in *Myristica malabarica* leaf oil.

Swapnil Balasaheb Patil et.al 2011 studied and reported the Spices are extensively used to enhance the taste and flavor of foods and are known to possess several medicinal properties. *Myristica fragrans*, *Parmelia perlata*, *Illicium verum*, *Trachyspermum copticum* and *Myristica malabarica,* the commonly used spices in India were assessed for antidiabetic activity in streptozotocin induced diabetic rats. In the *in vitro* insulin secretion studies on isolated islets of Langerhans, *M. fragrans, T. copticum and M. malabarica* showed dose dependent insulin secretion. *M. malabarica* showed the highest flavonoid content (i.e., 38.35 mg quercetin equivalents/g). Regular use of these spices may prevent postprandial rise in glucose levels through inhibition of intestinal alpha-glucosidase and may maintain blood glucose level through insulin secretagogue action.

Talukdar et.al 2000 Investigated the phytochemical constituents of an isoflavone from myristica malabarica and reported the isolation of the new 7,4′-dimethoxy-5-hydroxyisoflavone together with two other isoflavones, biochanin A and prunetin, and a 1,3-diarylpropanol and a rare α-hydroxydihydrochalcone.

3. PLANT PROFILE

MYRISTICA MALABARICA

DESCRIPTION

Family	: Myristicaceae
Kingdom	: Plantae
Genus	: Myristica
Species	: *Myristica malabarica*
Habit	: Tree
Habitat	: Evergreen Forests
Altitude	: 100 -800m
Higher classification	: Nutmeg

Vernacular Names

Malayalam -kattujathi

Hindi -Van jayaphal

Kannada -Kanage,Dhodda jaye kaayi

Telugu -Rampatri

Tamil -Pathiri,Kattu jaathi kaai

Sanskrit - Malati

DISTRIBUTION

Endemic to Western Ghats in southern states of Karnataka and Kerala. In Karnataka, occurs in evergreen forests of lower altitudes in Dakshina kannada, Uttara Kannda, Shimoga and Udupi districts. In Kerala, fairly common in the evergreen forests of lower Ghats. Not reported from Tamil Nadu.

DESCRIPTION

It is a medium sized dioecious evergreen tree, growing 10-17m tall and about 1.5m girth. Bark greenish-black, smooth, with projected lenticels, about 1 cm thick, partially fibrous, red inside, exuding deep red watery juice when cut fresh. Wood yellowish brown, tinged with grey, moderately hard. Branchlets hairless. Leaves alternate, linear-oblong or elliptic-oblong, 8-16x3-5cm, base rounded, apex acute, margin entire, hairless, glossy, leathery, distinctly stalked; lateral nerves 8-14 pairs, slender and faint.

About its flowers Male and female flowers seen in separate trees. Male flowers clustered at the end of branches of panicles, with slender stalks, 5-6mm long, creamy white, more or less hairless. Female flowers in small fascicles, ovoid, about 6mm long, dull yellow. Capsusles solitary or in pairs, cylindrcially oblong, 5-9x3-5cm, brown tomentose. Seeds single, completely covered by golden yellow or red-cloured fleshy aril.

TRADE DETAILS

Local and regional. A possible adulterant to Myristica fragrans. The mace of Myristica malabarica commercially known as Rampattari is often adulterated with the true Jaatipatri (Myristica fragrans). Kernels are sometimes mixed with those of M. fragrans, the true source of Jaaiphal.

SPECIAL FETURES

The bark exudes deep red watery juice when cut. Fruits are cylindrical, brown tomentose, splitting vertically by 2 valves, exposing seeds completely covered with brightly coloured dissected aril.

MODE OF PROPAGATION

By seeds and stem cuttings

MEDICINAL USES

Aril of the seeds is used to check cough, bronchitis, fever and burning sensation. Fat obtained from the seeds relieves pain in muscles, sprains and sores.

Myristica malabarica Houtt) is an evergreen thick shady tree which produces high commercial value nuts and aril (mace).Being known for its wide coverage of use in Pharmaceutical as well as nonPharmaceutical, we thought of having an insight to its full potentiality. On literature review we came to know that the fruit-seed structure is not that simple; at a point we were jumbled to differentiate the seed part in its dry form. Since gathering farming practice directly from the field gives a live observation, we decided to collect the specimen directly from a plantation and with the help of a botanist identify the parts. Nakshatra Extract Life Sciences (NELS) have its organic farm where trees are being cultivated as a major crop as an intercropping between coconuts. Other spices including pepper and many other fruit yielding trees added value to the farm which follows good farming practice (GFP) under experienced technical hands. The details regarding cultivation, collection, processing and products of were procured directly from their botanist, also from Pharmacopoeias, various

literatures and online data. The information available on the Pharmacognostical, Pharmacological, and Chemistry of (Myristica malabarica) has been extensively reviewed including details about cultivation parameters and usage.

Myristica malabarica Houtt, commonly known as Jathikka and Javitri in India, belongs to the family Myristicaceae. The name 'Myristica' is derived from the Greek word 'Myron', a sweet liquid distilled from the plant. M.malabarica is one of the aromatic plants that are endowed with alluring properties of fragrance and flavours and produces odoriferous secondary metabolites in their fruits. No wonder, the and mace have been popular for several hundreds of years. Geographical source Originated from the Banda Islands in the Moluccas of Eastern Indonesia, it is seldom found truly wild. It is now cultivated in tropical regions, especially Grenada in the West Indies, Sri Lanka and India Biophysical limits The plant grows at an altitude of 700-4500m with a temperature 25-30ºC. It requires a rainfall of 2000-3500mm. can grow on any kind of soil provided there is sufficient water but without any risk of water logging. It prefers soils of volcanic origin and those with high contents of organic matter with pH 6.5-7.5. Pharmacognostical details of the plant It is a spreading aromatic evergreen tree usually growing to 5 to 13 metres high, occasionally 20 metres. The pointed dark green leaves are arranged alternately along the branches and are borne on leaf stems about 1 cm long. Upper leaf surfaces are shiny. Flowers are usually single sexed; occasionally male and female flowers are found on the same tree. Female flowers arise in groups of 1 to 3; males in groups of 1 to 10. Flowers are pale yellow, waxy, fleshy and bell-shaped. The fruits are fleshy, drooping, yellow, smooth, 6 to 9 cm long with a longitudinal ridge. Fruit is yellow in colour having a shiny outer coat (exocarp) and a fleshy mesocarp below. Exocarp, mesocarp and endocarp together comprises the pericarp of the fruit. When ripe, the succulent yellow fruit (mesocarp) splits into 2 valves revealing a purplish-brown, shiny seed () surrounded by a red aril (mace). Seeds (s) are broadly ovoid (2 to 3 cm long), firm, fleshy, brownish-white and transversed by red-brown veins. is the seed kernel inside the fruit and mace is the fleshy red,net like skin covering (aril) over the kernel. Oil seed is within the seed coat (endocarp) which gets detached after drying. Testa and tegmen are the layers of seed while perisperm have oil ducts within the

endosperm which also houses embryo. When fresh, the aril (mace) is bright scarlet becoming more horny, brittle and a yellowish-brown colour when dried (Purseglove, 1968). The trees do not give flowers until around 9 years old, but once start flowering they continue to do so for further 75 years. The trees bear 2 to 3 crops a year. The seeds (s) need 3 to 6 weeks to dry before they are ready for use. Digital pictures representing various parts of yield and its artistic illustration is shown in figure 1 and 2 respectively. Figure 2 E and F shows partly removed calyx of male and female flower.

Cultivation, processing and harvesting Young plants should be planted under 50% shade, but can be reduced progressively and after 6-7 years they can grow without shade at all. Trees should be well spaced so that branches don't touch each other and not to hamper flowering. Lower branches should be pruned to facilitate collection of dropped seeds. On our visit to organic farm of 'Nakshtra extracts Life Sciences' we were informed by their skilled resource person that as the tree grows the tap root degenerates and the fibrous roots become more active superficial feeders which provides the buoyancy for the entire tree. 1.5. Propagation The main problem is segregation of seedlings into male and female plants, resulting in the production of 50% unproductive male plants. So it's better to adopt budded or grafted plants. Grafted plants are planted into the main field during the beginning of rainy season. Pits of 0.75m×0.75m×0.75m size are dug at a space of 9m×9m and are filled with organic manures and soil 15 days prior to planting. A male graft has to be planted for every 20 female plants in the field. Plants should be shaded in order to be protected from sun. It can be best when grown as ntercrop in coconut gardens which are more than 15 years old. 1.6. Manures and fertilisers Information collected from organic farm of Nakshtra extracts Life Sciences recommends that care should be taken to avoid both water logging and drying of the soil since the tree is hydro sensitive. Ideally, 3-4 water sprinklers provide optimum watering during summer. Many farmers' supply bone meal (for calcium), goat droppings/ dried cow dung to base without disturbing roots which are superficial feeders. Levigation of mud on top of the manure helps its easy digestion.

Harvesting The female trees start fruiting from 6 years old even though peak period is 20 years. The peak harvesting season is June–August. The fruits are ready for harvesting when the pericarp splits open. After harvesting outer fleshy part is removed and the mace is removed from the nut followed by sun drying. There are people who practice drying over native wood fire stove, especially during monsoon. The scarlet coloured mace becomes yellowish brown and brittle when drying is completed. The fleshy pericarp can be used for making jams, pickles and jellies. 1.8. Varieties Indian Institute of Spices Research has released 'IISR vishwasree' which yield about 1000 fruits at the 8 th year of planting. IISR has also released few elite lines such as A9- 20, 22, 25,69,150, A4-12,22,52, A11-23,70 as high yielders and distributed as grafts. 1.9. Pests and diseases The most serious pest is the scolytid beetle Phloeosinusribatus which bores through bark causing dieback and death. Other damaging borers are Xyleborusfornicatus and X. myristcae. The coffee bean weevil Ataecerus fasciculatus is a serious pest of stored and mace. The only fungal disease of major importance is Stigmina myristicae, a dry rot that causes the fruits to open when still young. Consequently the arils and seeds remain underdeveloped and are worthless. Soft rot of fruits caused by Colletotric humgloeosporioides also causes young unripe fruits to open prematurely. Root rots caused by Fomesnoxius and Fomeslamaoensis may cause considerable damage. While most of the farmers depend on 1% bordeaux mixture (CUSO4: lime: water in the ratio of 1:1:100) for fungal rots, NELS practice 1% Pseudomonas solution as spray which is beneficial to preserve proper soil biosystem. It is mandatory to maintain the field neat and tidy to avoid plant diseases and mosquitos which may be attracted by water filled split fruit coat. Many farmers supply neem cake to the tree base. 1.10. Chemical composition of Myristica malabarica fruit The seed contains about 10% essential oil (Verghese 2001; Maya et al. 2004), which is mostly composed of terpene hydrocarbons (a-pinenes, camphene, p-cymene, sabinene, b-phellandrene, gterpinene, limonene, myrcene (60% to 90%), terpene derivatives (linalool, geraniol, terpineol-5% to 15%) and phenylpropanes (myristicin, elemicin, safrole-2% to 20%). The presence of myristicin and elemicin, in the seed of M. malabarica is one of the reasons for its intoxicating

effects has made extensive studies on the composition of and mace. The seeds also contain 25-30% fixed oils (myristic, stearic, palmitic, oleic, linoleic and lauric acids).

4. AIM AND OBJECTIVE

Cancer is a one of the life threatened disease in this world and now a days which may appeared worldwide problem. As per the literature survey, globally one in five men and one in six women will develop cancer before the age of 75 and one in eight men, and one in twelve women, will die from the disease. Nearly seven lakh Indians die of cancer every year, while over 10 lakh are newly diagnosed with some form of the disease. Several modern day synthetic drugs are available in market do not full fill the requirements with side effects like allopecia, cytotoxicity, bone marrow depression etc. So the priority is drugs which minimize the side effects and also require a chemical entity for the treatment of cancer. Several literatures indicated traditional herbs possessing lesser side effects compared to synthetic drugs. The herbal formulations were developed from Ayurveda, traditional system of Indian medicine and its additional systems of medicine, has been found to have anticancer activity. Some of the research workers published has been published my study related species like *Myristica Fragrance* possess anti-cancer activity. *Myristica Malabarica* is a traditionally used herb but there is no scientific validation which old version of books and oliaichuvadi (Palmleaf petal writings) anti angiogenesis activity. Based on the above information's I was selected the plant, perform the extraction and current work aims for identification of anticancer constituents from the fruits of *Myristica Malabarica* and evaluated by using some *in vitro* technique.

5. PLAN OF WORK

- Collection and authentification of the genuine plant material from its natural source.
- Extraction of the plant material using ethyl acetate in reflux apparatus name of the apparatus.
- Extraction of the dried seeds of the *myristica malabarica* by using alcohol, chloroform, petroleum ether, ethyl acetate and water.
- Preliminary screening of the ethyl acetate extract of the plant using specific chemical tests.
- To perform thin layer chromatography to be find out the nature of volatile and solubility of compounds by using different solvent system.
- Anti angiogenesis study using different extracts of *myristica malabarica*.
- Selection of the fraction with higher activity.
- In vitro cytotoxicity study (MTT ASSAY) by using cancer cell lines.
- Identification of the chemical compounds present in the active fraction using LCMS analysis.
- Prediction of biological activity of each constituent by using Bio-informatics software PASS.
- Conforming the predicted anti cancer activity of active constituents from literature.
- Conclusion of the work.

6. MATERIALS AND METHODS

6.1 Collection of the plant

Dried plant materials were collected from the Raw Drug Maker. Collected plant material was then shade dried and powdered.

Requirements

Instruments

- Chemicals
- Solvents
- Drugs
- Cells

6.2 Extraction of Plant Material

Extraction:1

About 1kg of the powdered sample was weighed and extracted with 1000ml ml of Ethyl acetate (Merck, Germany) by reflux method. After the reflux, extract was collected dried under reduced pressure, which stored in desiccators. Complete moisture was removed from extract, which used for preliminary Phytochemical Screening, Thin Layer Chromatography, LCMS and *invitro* studies**.(write any one method of extraction).**.

Extraction:2

About 2kg of the plant materials was extracted with 1000ml methanol (Merck, Germany) using Reflux method. The extract was then cooled, filtered and concentrated to dryness. It was then dissolved in 100 ml methanol. This extract was then subjected to Thin Layer Chromatography.

- If the response to the test is high it can be noted as +++which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.
- If the response is very small then note it as + indicating the presence of only in traces.

- If no response is then negative.

6.3 PRELIMINARY PHYTOCHEMICAL SCREENING OF THE PLANT

The Ethyl acetate leaf extract of was used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates

- Molisch's test: Dissolved small quantity of 300mg alcoholic and dried leaf extract powder of Pimenta dioica separately in 4ml distilled water and filtered. The filtrate was
- Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution
- Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.

Test for flavonoids

Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added ..Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added.

Test for tannins

- Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added.

Test for steroid/terpenoid

- Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

Test for alkaloids

- Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent

- Hager's test: The extract was treated with few ml of Hager's reagent.
- Wagner's test: The extract was treated with few ml of Wagner's reagent. **Tests for Glycosides**
- Legal's test: Dissolve the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution

Test for Saponins

- Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.

Test for Anthraquinones

- Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.

Test for Amino acids

- Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent

Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper.

6.4 ANTI ANGIOGENISIS STUDY

The *Myristica Malabarica* different extracts are suspended by 0.5% carboxy methyl cellulose which was used for the *invitro* studies . For each series of strength required 5 country eggs were taken per group. Each egg should be hold the vertical manner and oval portion at the upper side has cut by using a surgical blade a small portion of the upper part of the egg is cut open in a round manner, without loss of the outer membrane. Accurately diluted forms of extract were transferred by using small capillary tube insert into the egg and slowly administer by syringe and the open part is close with the membrane coated portion of the egg. The opened portion of the egg is

tightly close to prevent the air contamination into the egg, which was kept in a aseptic condition. Similarly the each concentration of the extracts were done in a same manner. .Totaly 25 eggs are use for the analysis. The solvent, drug and extract treated eggs were kept in a incubation period of 21 days for incubator. .After 21st day the egg is cut open and kept the content in a petridish and analyse the anti angiogenesis activity.

6.5 INVITRO CELL LINE STUDY (CYTO TOXICITY STUDY)

MTT ASSAY

MTT Assay is a type of calorimetric assay which involves the measurement of the enzyme in the cells which converts dye MTT to blue forzaman(refer). Main application of this method was to determine the viability and proliferation of the cells. It is mainly used to determine the cytotoxicity of the medicinal agents such as plant extract. The main disadvantage is it can be only applied to biological cells .

CELL LINES AND CULTURE MEDIUM

HCC 2935 and HCC4006 cell lines (human lung cancer) cell line culture were produced from National centre for cell lines(NCCS). pune, india. Stock cells of HCC2935 and HCC4006 cell lines were cultured in DMEM supplemented with 10% inactivated fetal bovine serum(FBS), penicillin (100IU/ml),streptomycin(100mcg/ml),and amphotericin B(5mcg/ml),in humidified atmosphere of5%CO2 at 37C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin,0.02%EDTA,0.05%Glucose in PBS).The stock culture were grown in 25cm^2 culture flasks and all experiments were carried out 96 microtitre plates (Tarson India Pvt Ltd.,Kolkata.India).

PROCEDURE

Preparation of of test solutions

For toxicity studies weighed test drug were dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated foetal bovine serum to obtained a stoke solution of 1 mg/ml concentration and sterilised by

filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Assay procedure

Step 1: Adjustment of cell count:

The monolayer of cell culture was trypsinized and the cell count was adjusted with **DMEM** medium containing 10% FBS to$1.0*10^5$ cells/ml.

Step2: Addition of cell suspension:

To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension was added.

Step3: Addition of Test Compound

After 24hrs, partial monolayer was formed, then supernatant was flicked off,and the monolayer washed once with medium. Then 100mcl of different test concentration of test compound were added on to the partial monolayer in microtitre plates.

Step4: Incubation Period:

The plates were then incubated at 37^0 c in 5% co_2 atmosphere, and microscopic examination was carried out and observation were noted every 24hrs of interval.

After 72hrs,the drug solution in the wells were discarded and 50mcl of MTT in PBS was added to each well.

Step 6: Incubation Period:

The plates were shaken gently and incubated for 3hrs at 37^0 c in co_2 atmosphere.

Step 7: Separation of Forzaman:

The supernatant was removed and 100mcl of propanlol was added and the plates were gently shaken to solubilise the forzaman. The absorbance of the solution obtained was measured using a microplate reader at a wave length of 540nm.

Calculation

The percentage growth inhibition was calculated by using the following formula and concentration of test compound needed to inhibit cell growth by 50%(CTC_{50}) Values is generated from the dose-response curve for each cell.

Percentage growth inhibition= 100-OD of individual test group*100

OD Mean value

6.6 METHODOLOGY FOR THE LC-MS ANALYSIS

LC-MS has become method of choice in many stages during drug development process. Recent advances includes electrospray, thermospray, and ionspray ionization techniques which offer unique advantages of high detection sensitivity and specificity, liquid secondary ion mass spectroscopy, later laser mass spectroscopy with 600 MHz offers accurate determination of molecular weight proteins, peptides. Isotopes pattern can be detected by this technique. For the most recent LC-MS on the market, an automatic procedure is included in the software package to tune and calibrate in the ESI mode. However, olderinstruments and/or very specific applications still require manual or semiauto-matic procedures to optimize the parameters that affect ion detection. In anLC-MS instrument, the mass spectrometer is tuned and calibrated in three steps:(1) ion source and transmission optimization, (2) MS calibration, and (3) fine tuning. As per the above specified standard procedure based to set specifications such as LC Column: Reverse Phase C-18, Pump: SPD 10 AVP, Mobile Phase: Water: Methanol: THF (50:40:10), Ionisation Mode: Electronic Spray Ionization, Mode: Both Positive, Injection Volume: 10 Micro litre, Flow rate: 1.5 ml/min, Column temperature: 25^0c, Column: PHENOMENEX RP 18, Column dimension: 25 cm x2.5 mm, LC Detection: 264 nm, M/Z Range: 50-1000 and Soft ware: Class V P Integrated and Library: Metwin 2.0

6.7 PASS (Prediction of activity spectra of substances)

PRINCIPLE

PASS is the computer generated program which provides the simultaneous prediction of several hundreds of biological activity types for any drug-like compounds. In this bio informatics software prediction is based on the analysis of structure-activity relationships of (SAR) the training set including more than 30000 known biologically active compounds. In this paper mainly investigate the influence on the accuracy of predicting the types of activity with PASS by (a) reduction of the number of structures in the training set and (b) reduction of the number of known activities in the training set. Here mainly demonstrate that predictions are robust despite the exclusion of up to 60% of information .The compounds from the MDDR database are used to create heterogeneous training and evaluation sets .

PROCEDURE

To estimate the activity spectrum for a new compound (C) its MNA descriptors are generated. For each type of activity (*j*) the value of *tjC* is calculated. The probabilities of presence Pa*j* and absence Pi*j* of *j*-th activity type in the compound are calculated according to the next equations *Aj*(Pa)) *tj*C; *Ij*(Pi)) *tj*C In other words, Pa and Pi are the probabilities of belonging to the classes of active and inactive compounds, respectively. The result of prediction for a new compound is the *activity spectrum,* which is the ranked list of activity types with estimated Pa and Pi values. The ranking is executed on descending order of Pa-Pi; thus, more probable activity types are at the top of predicted spectrum. Compound is considered as active if Pa-Pi exceeds the cut off value. By default here use cut off of Pa - Pi 0, but any user may accept his own cut off value.

7. RESULT AND DISCUSSION

Class of compounds	Tests performed	Results
Carbohydrates	Molisch's test	+
	Fehling's test	
Phenols	Phosphomolybdic acid test	+++
Flavonoids	Shinoda test	++
	Lead acetate test	
Tannins	Braemer's test	_
Alkaloids	Wagner's	+
	Mayer's	
	Draggendrof's test	
Glycosides	Legal's test	+
	Brontranger's test	
Saponins	Foam test	_
Sterols	Salkowski's test	++
Aminoacids	Ninhydrin test	_
Terpenoids	Libermann Burchardt test	+

The phytochemical studies results revealded that the Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates. Blue coloration of the spot indicated the presence of phenols. A pink or red coloration of

the solution indicated the presence of flavonoids in the drug. Flocculent white precipitate indicated the presence of flavonoids. Dark blue or greenish grey coloration of the solution indicated the preseence of tannins in the drug. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids. Orange coloration of the spot indicated the presence of alkaloids. Yellow precipitation indicated the presence of alkaloids. The reddish brown precipitation indicated the presence of alkaloids. Pink to red color solution indicates the presence of glycosides. A1cm layer of foam formation indicates the presence of Saponins. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones. Blue color indicated the presence of amino acids. Oil stains on the paper indicated the presence of fixed oils. If the response to the test is high it can be noted as +++which indicates that the particular group is present as the major class. If the response is average then note it as ++ indicates the presence in moderate quantity. If the response is very small then note it as + indicating the presence of only in traces. If no response is then negative.

NB:--- indicate not present

\+ In traces

++ present in moderate amount

+++ more amount is present

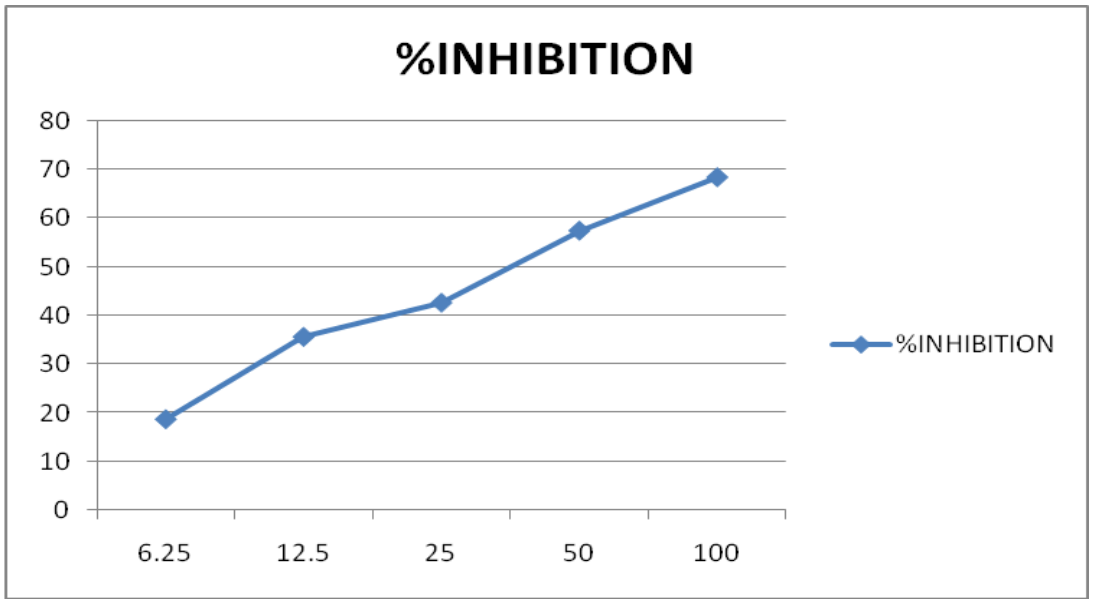
IN VITRO CELL LINE STUDY (MTT ASSAY)

Cytotoxicity Effect Of myristica malabarica on colon cancer cell lines

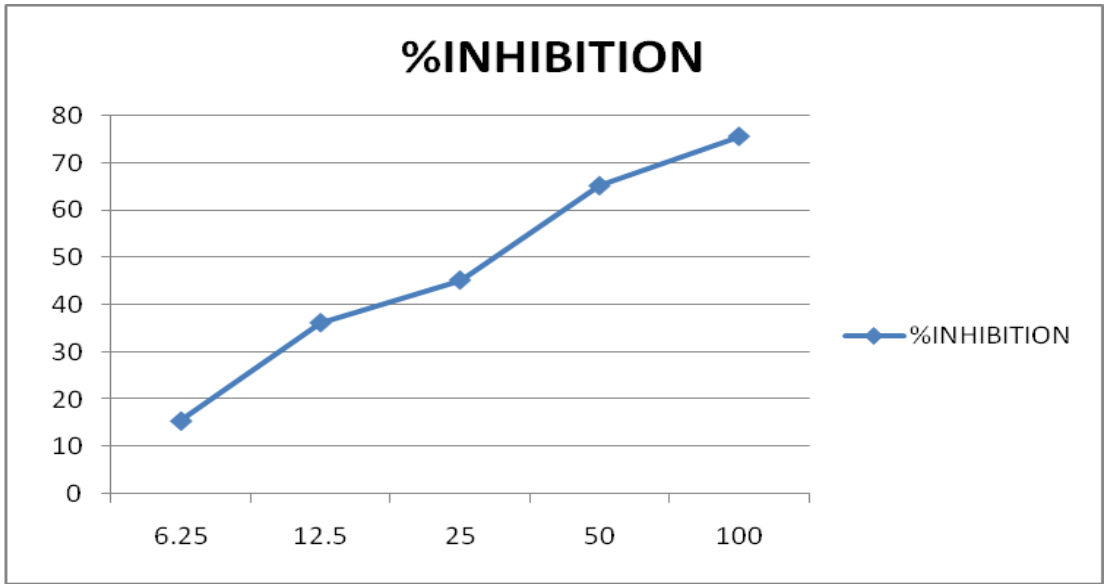
SI NO	Cell Lines	Name Of Drug	Conc(mcg/ml)	% Inhibition	Average CTC_{50}(mcg/ml)
1.	HCC2935	MYRISTICA MALABARICA	6.25 12.5 25 50 100	18.72±0.59 35.6± 2.8 42.6± 1.82 57.36± 5.04 68.3± 4.16	44.51
2.	HCC4006	MYRISTICA MALABARICA	6.25 12.5 25 50 100	15.45± 1.55 36.2± .35 45.2± 0.68 65.1± 1.1 75.2 ± 1.55	47.43

The mean ± standard error values were expressed which evaluated by one way ANOVA followed by Dunnet 't' test

Cytotoxicity effect of myristica malabarica extract on HCC2935 Cell lines



Cytotoxicity effect of myristica malabarica extract on HCC4006 Cell lines



TLC RESULTS OF *MYRISTICA MALABARICA*

Si No	Solvents	Concentration	RF Value
1.	Toluene+ Ethyl Acetate	7:3	0.53
2.	Ethyl Acetate+ Toluene +Glacial Acetic Acid	5:5:1	0.66
3.	Petroleum Ether+ Chloroform	7:3	0.62
4.	Ethyl Acetate+ Methanol	1:1	0.46
5.	Hexane+ Dichloro Methane	1:1	0.47
6.	Ethyl Acetate+ Methanol	3:1	0.45
7.	Dichloro Methane+ Hexane	3:1	0.56

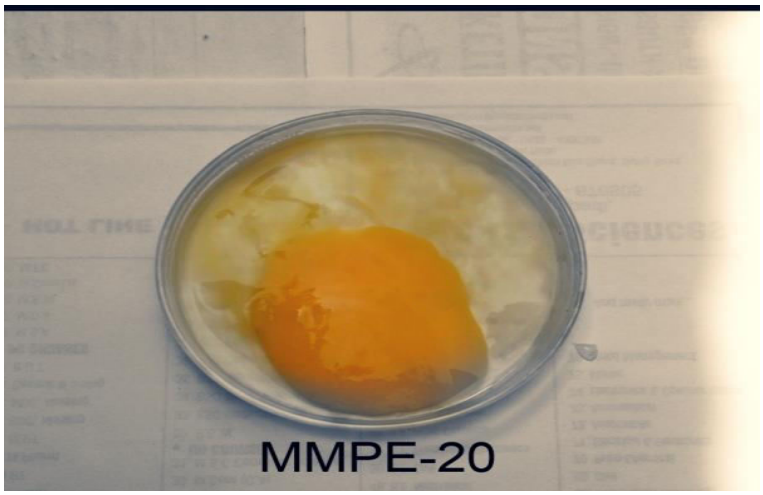
RF Value range 0-1-High polar substances

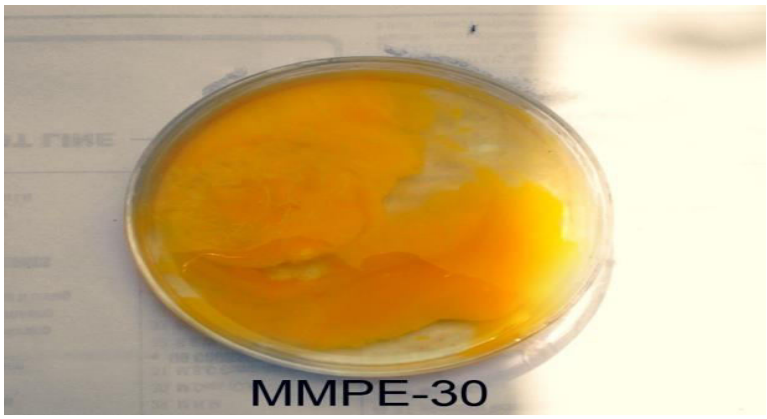
Low RF Value- Higher polar substances may present

Higher RF Value- Low polar substances may present

Solubility of the compounds is directly proportional to polarity of the compounds.

Myristica malabarica **petroleum ether extract**

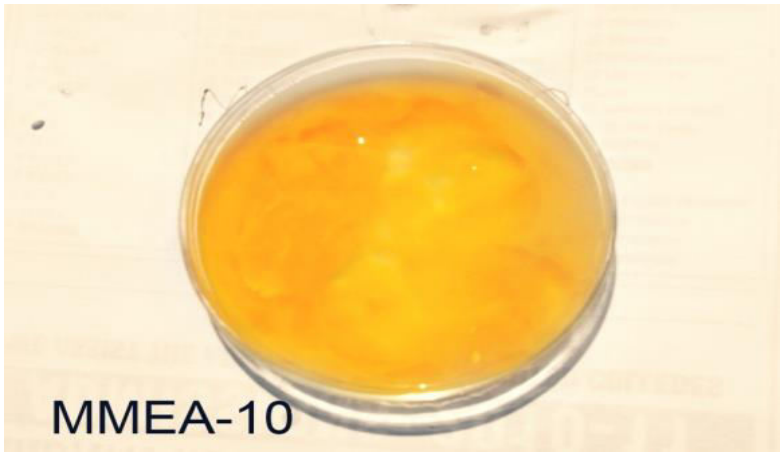


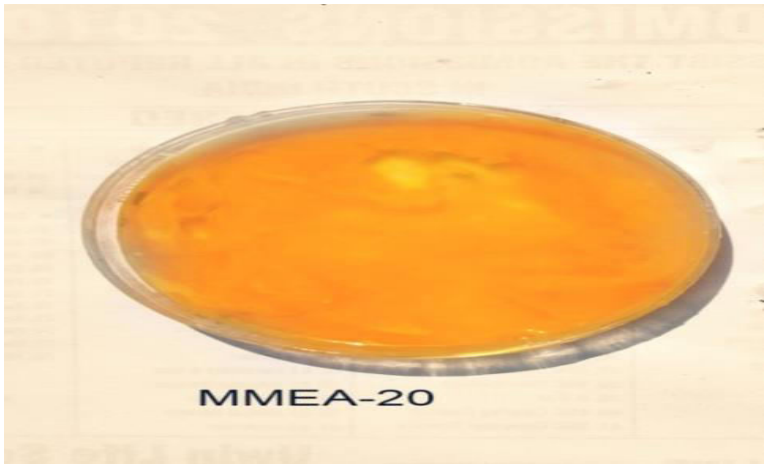


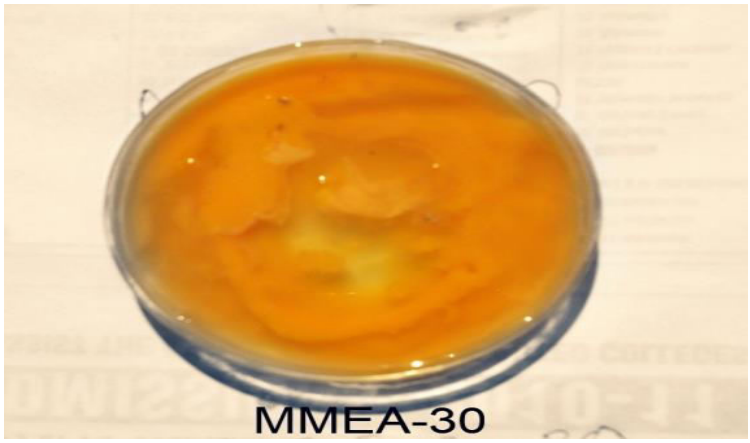
MMPE: Myristica malabarica Petroleum Ether Extract.

10, 20, 30: mcg concentration

Myristica malabarica **Ethyl acetate extract**



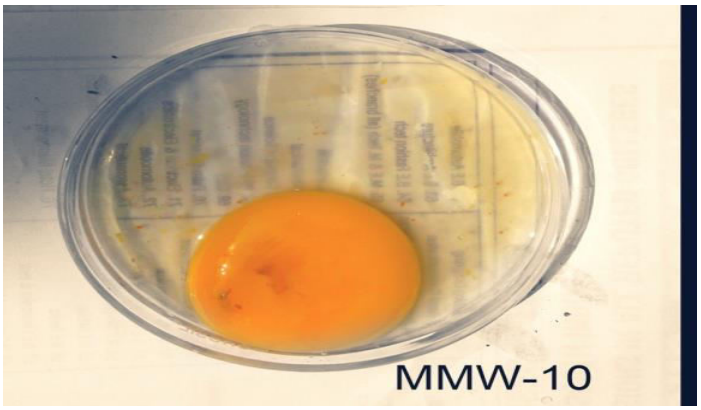


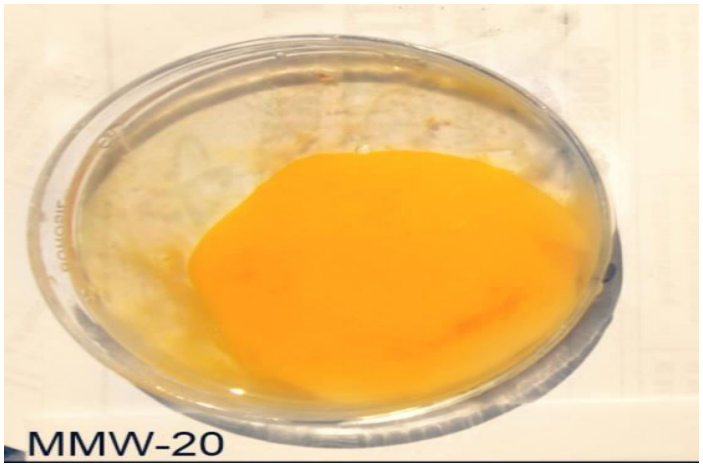


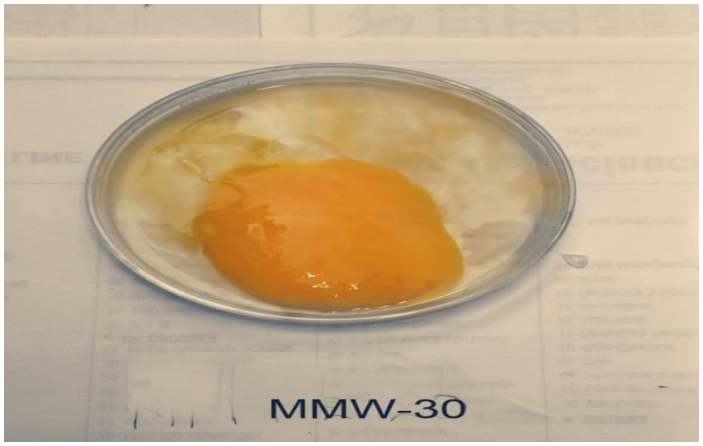
MMEA: Myristica malabarica Ethyl Acetate Extract

10, 20, 30: mcg concentrations

Myristica malabarica **Water extract**



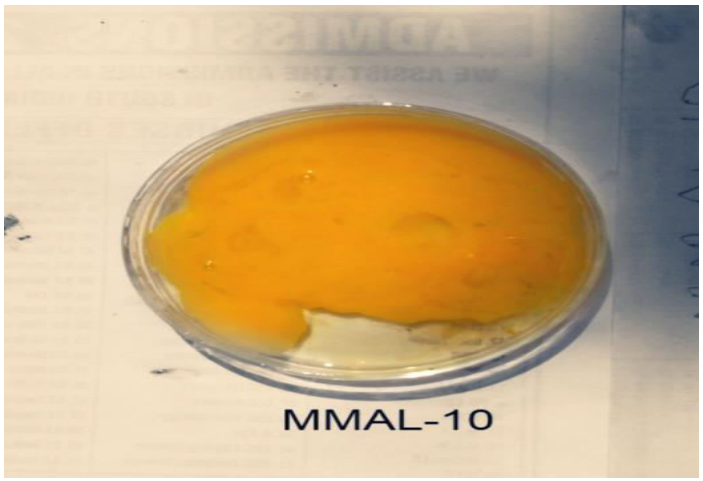


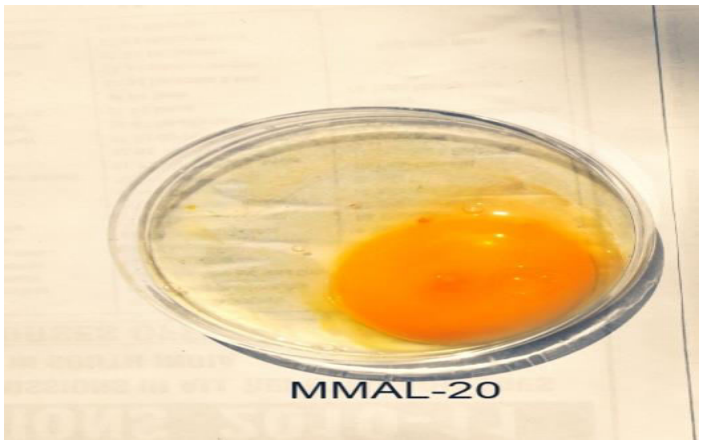


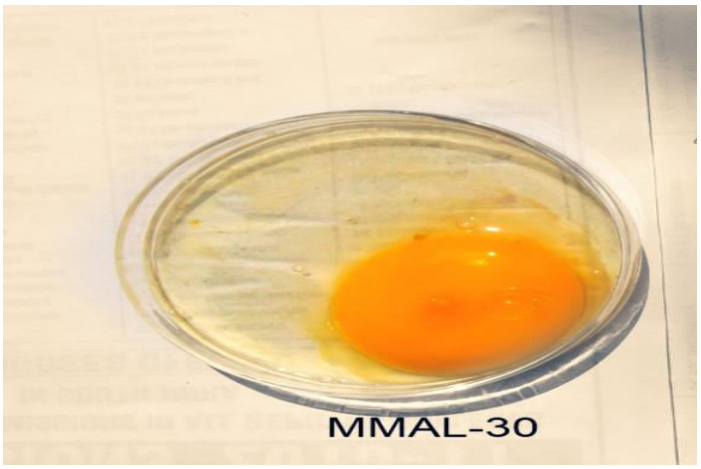
MMW: Myristica malabarica Water Extract

10, 20, 30: mcg concentrations

Myristica malabarica **Alcohol extract**



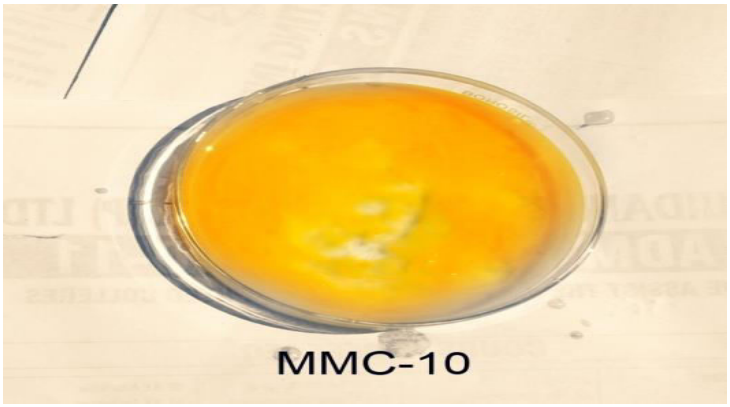


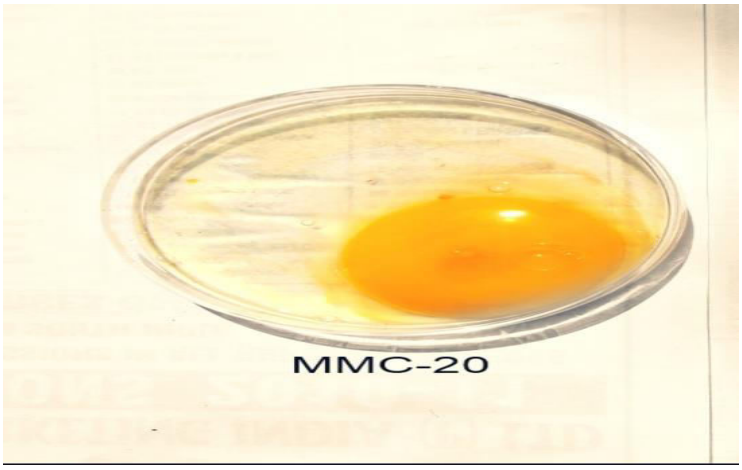


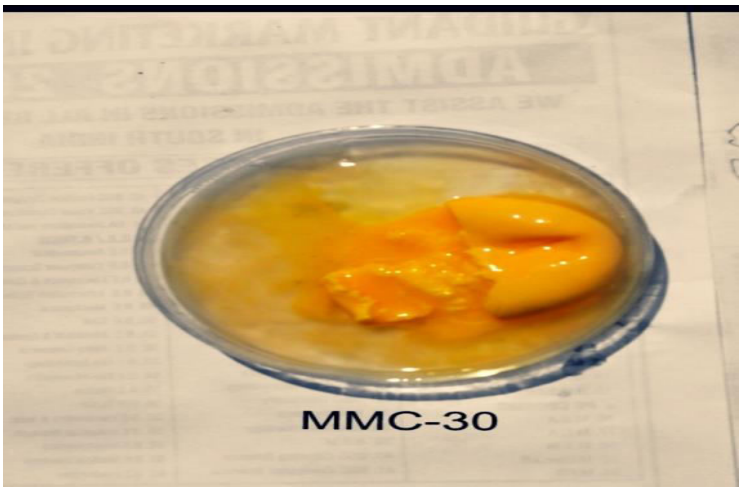
MMAL: Myristica malabarica Alcohol Extract

10, 20, 30: mcg concentration

Myristica malabarica **Chloroform extract**







MMC: Myristica malabarica Chloroform Extract

10, 20, 30: mcg concentration

8. DISCUSSION AND CONCLUSSION

Angiogenesis is a hallmark of tumor development and metastasis and is now a validated target for cancer treatment. However, the survival benefits of anti-angiogenic drugs have, thus far, been rather modest, stimulating interest in developing more effective ways to combine anti-angiogenic drugs with established chemotherapies. New targets for anti-angiogenesis continue to be discovered, increasing the opportunities to interdict tumor angiogenesis and circumvent resistance mechanisms that may emerge with chronic use of these drugs.

The control of tumor angiogenesis is an integral part of the host defense response to tumor growth. Loss of endogenous angiogenesis inhibitors, such as endostatin and thromobospondin-1, leads to increased tumor angiogenesis and accelerates tumor growth. The inhibition of tumor growth by anti-angiogenic drugs has been achieved both in preclinical studies and in clinical trials, where promising anti-tumor responses have been reported for a variety of anti-angiogenic agents

The survival benefits of anti-angiogenic drugs have, thus far, been rather modest, leading to increased interest in developing more effective ways to combine anti-angiogenic drugs with traditional, cytotoxic chemotherapies. In this review, we discuss recent progress and some emerging challenges in the development of anti-angiogenic drugs for cancer treatment.

Tumors need to develop their own blood vessels in order to survive, proliferate and invade other tissues, making antiangiogenesis an interesting target for antitumor therapy. Treatments can be direct, targeting membrane receptors, indirect, targeting angiogenic factors or both.

Using chick CAM model ,the new pharmacological effect of Myristica malabarica extract have been confirmed by the proven inhibition of angiogenesis. It showed that significant antiangiogenic activity of all hours of treatment studied and extract studies.

The antiangiogenic property of Myristica malabarica may be attributed to the phytochemical present in the plant .It could be function of either the individual or the additive effect of phytoconstituents may be phenol, terpinoids, and flavanoids have shown to inhibit carcinogenisis and tumerogenisis.

From qualitative phytochemical analysis the presence of alkaloids ,flavanoids and terpinoids may be atributed to the potential antiangiogenic property . The strong antiangiogenic properties of this plant support the ethnomedical claim of the Myristica malabarica.

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